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PREVALENCE OF *SARCOCYSTIS* SPECIES SPOROCYSTS IN WILD-CAUGHT OPOSSUMS (*DIDELPHIS VIRGINIANA*)

J. P. Dubey

United States Department of Agriculture, Agricultural Research Service, Livestock and Poultry Sciences Institute, Parasite Biology and Epidemiology Laboratory, Beltsville Agricultural Research Center, Beltsville, Maryland 20705-2350

ABSTRACT: *Sarcocystis* sporocysts were found in intestinal scrapings from 24 (54.5%) of 44 opossums (*Didelphis virginiana*). The number of sporocysts varied from a few (<10,000) to 245 million. Sporocysts from 23 of 24 opossums were fed to captive budgerigars (*Melopsittacus undulatas*), and the inocula from 21 opossums were infective, indicating the presence of *Sarcocystis falcatula*. Sporocysts from 24 opossums were fed to gamma-interferon-knockout (KO) or nude mice; inocula from 14 opossums were infective to mice. *Sarcocystis neurona* was detected in tissues of KO mice by specific staining with anti-*S. neurona* antibodies, and the parasite was cultured in vitro from the brains of KO mice fed sporocysts from 8 opossums. *Sarcocystis speeri* was identified by specific staining with anti-*S. speeri* antibodies in tissues of KO mice fed inocula from 8 opossums; 3 opossums had mixed *S. neurona* and *S. speeri* infections. Thus, the prevalences of sporocysts of different species of *Sarcocystis* in opossums were: *S. falcatula* 21 of 44 (47.7%), *S. neurona* 8 of 44 (18.1%), and *S. speeri* 8 of 44 (18.1%) opossums. *Sarcocystis neurona* alone was found in 1 opossum, and *S. speeri* alone was found in 1 opossum. Mixed *Sarcocystis* infections were present in 21 opossums.

The North American opossum (*Didelphis virginiana*) is the definitive host for at least 3 pathogenic species of *Sarcocystis*: *Sarcocystis neurona*, *Sarcocystis falcatula*, and *Sarcocystis speeri* (Box et al., 1984; Dubey et al., 1991; Fenger et al., 1997; Dubey and Lindsay, 1998, 1999; Dubey et al., 1998). The objective of the present investigation was to determine the prevalence of *Sarcocystis* species in opossums because there is little information on this subject.

MATERIALS AND METHODS

Sporocyst collection

Small intestines from naturally infected opossums were obtained from various sources, including some road kills collected January 1998 to June 1999 (Table I). Of the 44 opossums surveyed, 9 were from the Audubon Park and Zoological Garden, New Orleans, Louisiana; 16 from the Metro Zoo, Miami, Florida; 11 from areas surrounding Beltsville, Maryland; and 8 from other states (Table I). The small intestinal mucosa was scraped with a slide and scrapings were homogenized in 100 ml of water for about 30–60 sec in a domestic blender at top speed. One drop of the homogenate was examined microscopically for sporocysts. The sample was not processed further if no sporocysts were seen. After rinsing the blender jar, the volume was made up to 1,000 ml with water to which a few drops of detergent (Tween 80 or any other detergent) were added. The mixture was shaken for 1–2 hr at room temperature (22 C), filtered through gauze, and then centrifuged at 1,000 rpm (~600 *g*) for 10 min. After discarding the supernatant, the sediment was suspended in ~100 ml of water and examined microscopically to ensure that intestinal cells were disrupted. If intestinal villi were not disrupted, the sediment was rehomogenized in a blender and the process repeated. After final centrifugation at 2,000 rpm (~1,200 *g*), sediment was suspended in Hanks' balanced salt solution (HBSS), filtered through a 90-μm metallic sieve, and the filtrate was centrifuged at 2,000 rpm. The final sediment was suspended in 25 ml of an antibiotic solution (penicillin, 10,000 U; streptomycin, 10 mg; fungizone, 0.05 mg, and mycostatin 500 U/ml) in HBSS. Sporocysts were counted in a hemacytometer and stored at 4 C.

Bioassay of sporocysts in birds and mice

Budgerigars (*Melopsittacus undulatus*) and immunodeficient mice were used to distinguish sporocyst species in opossums (Dubey and Lindsay, 1998). Organisms infective to budgerigars were considered *S. falcatula* because *S. neurona* and *S. speeri* are not infective to budgerigars (Marsh et al., 1997; Dubey et al., 1998; Dubey and Lindsay, 1998, 1999). The budgerigars were obtained from a local aviary and were housed individually or in groups of 2.

In the beginning of the investigation, only nude mice (C57BL/6JHFHll-Nu) were used to test infectivity of sporocysts based on the study of Marsh et al. (1997). When it was discovered (Dubey and Lindsay, 1998, 1999) that gamma interferon knockout (KO) mice (BALB/c^{Ifng}) were also susceptible to *S. neurona* and *S. speeri* infection, they were used preferentially (Dubey and Lindsay, 1998, 1999) (Table II). Nude and KO mice were obtained from Jackson Laboratories, Bar Harbor, Maine.

Each animal (budgerigar or mouse) was given orally ~1/50th of the total number of sporocysts shown in Table I. The birds were observed for 33 days, whereas mice were kept up to 90 days. Bedding and floor coverings in each bird or mouse cage were incinerated for 7 days postfeeding (DPF) to kill any sporocysts that might pass unexcysted in feces.

Histology

All birds and mice that died or were killed were necropsied. Samples of all major organs were fixed in 10% buffered neutral formalin and processed for histology (Dubey and Lindsay, 1998, 1999; Dubey et al., 1998). Impression smears of lungs of birds and livers and brains of mice were examined microscopically for merozoites and schizonts after staining with Giemsa.

In vitro cultivation

An attempt to culture *S. falcatula* was made from 1 budgerigar fed sporocysts from opossum 8013 (Table I). For this, bovine monocyte (M617) cells were inoculated with lung homogenate of the budgerigar that was killed 6 DPF (Table I). The cultures were maintained as described by Lindsay et al. (1999). Attempts were made to culture organisms from the liver or brain of KO or nude mice that developed clinical disease after feeding sporocysts using procedures described previously (Dubey and Lindsay, 1998; Dubey, Mattson et al., 1999; Dubey et al., 2000).

Immunohistochemical staining (IHC)

Budgerigar tissues were reacted with anti-*S. falcatula* polyclonal serum from rabbits inoculated with the Cornell strain of *S. falcatula* (Dubey and Lindsay, 1998). The peroxidase–anti-peroxidose method was employed to reduce nonspecific background staining. Lungs from a bird that died after subcutaneous inoculation with culture-derived *S. falcatula* merozoites of the Cornell strain were used as a positive control (Dubey and Lindsay, 1998).

Tissues from mice were reacted with both anti-*S. neurona* and anti-*S. speeri* antibodies. Antisera against *S. neurona* were made in rabbits injected with culture-derived *S. neurona* merozoites originally isolated from opossum 95 (Dubey, Mattson et al., 1999). Antisera against *S.*

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TABLE I. Prevalence of *Sarcocystis* spp. sporocysts in opossums and infectivity to budgerigars.

Opossum no.	Source	Date	Total no. of sporocysts ($\times 10^6$)	*S. falcatula* infectivity to budgerigars: Day* died or killed	Schizonts	Sarcocysts
8013	Zoo, Louisiana	1-30-98	16	6	+	−
8026	Metro Zoo Florida	2-27-98	25	‡	−	−
8030	Manheim, Pennsylvania	2-19-98	2.5	6	+	−
8034	Beltsville, Maryland	2-23-98	20	10	+	−
8039	University Park, Pennsylvania	2-26-98	30	9	+	−
8047	Zoo, Louisiana	3-6-98	22.5	9	+	−
8052	Zoo, Louisiana	3-9-98	32.5	8	+	−
8064	College Park, Maryland	3-27-98	2.5	9, 19	+	−
8071	Leesburg, Virginia	4-19-98	1.25	9, 9	+	−
8074	College Park, Maryland	4-20-98	25	9	+	−
8080	Athens, Georgia	4-3-98	Few§	Not done		
8083	Zoo, Louisiana	3-5-98	100	5	+	−
8093	Metro Zoo, Florida	5-21-98	120	13	+	−
8095	College Park, Maryland	5-28-98	100	27, 27	−	−
8105	College Park, Maryland	6-9-98	245	7	+	−
8114	Zoo, Louisiana	6-30-98	Few§	10	+	−
8116	Zoo, Louisiana	7-15-98	Few§	9	+	−
8128	College Park, Maryland	8-10-98	70	8	+	−
8129	Zoo, Louisiana	8-13-98	Few§	k 34	−	+
9033	Metro Zoo, Florida	3-3-99	160	9, 9	+	−
9046	Montros, Virginia	3-29-99	70	8, 10	+	−
9047	Montros, Virginia	3-29-99	2	k 33, k 33	+	+
9062	Metro Zoo, Florida	4-16-99	70	13, 13	+	−
9108	Montros, Virginia	6-19-99	100	10, 10	+	−

* Day died or killed for each bird fed sporocysts; k = killed in good health, the remainder died or killed when ill.
† Audubon Park and Zoological Garden, New Orleans, Louisiana.
‡ Not infected (see Dubey and Lindsay, 1999).
§ Less than 10,000.

speeri were made in a rabbit against culture-derived *S. speeri* merozoites (Dubey and Lindsay, 1999).

RESULTS

Sarcocystis sporocysts were found in intestinal homogenates of 24 (54.5%) of 44 opossums, 7 of 9 from Louisiana, 4 of 16 from Florida, 6 of 11 from Maryland, and 7 of 8 from other areas (Table I). The total number of sporocysts in intestinal scrapings ranged from a few (<10,000) to 245 million; in 6 opossums there were ≥100 million sporocysts (Table I).

Sporocysts from 21 of 23 opossums were infective to budgerigars. Inocula from 19 opossums were lethal; budgerigars died 5–19 DPF. Budgerigars died suddenly without prior clinical signs. Schizonts were identified in the lungs of birds that were examined 5–33 DPF (Figs. 1–3). Sarcocysts were seen in budgerigars fed sporocysts from opossums 8129 and 9047 (Table I). More sarcocysts were seen in sections of tongue and leg muscles than in pectoral muscles.

Sarcocystis falcatula schizonts were found mostly in the lungs of infected budgerigars. Schizonts in lungs of all birds reacted positively with anti-*S. falcatula* antibodies (Fig. 3). More schizonts were identified by the IHC method than in hematoxylin and eosin (H&E)-stained sections. Sarcocysts from birds that were killed 33 and 34 DPF either did not stain or reacted faintly with *S. falcatula* antibodies in the IHC test.

Sarcocystis falcatula was cultured from a lung homogenate of the budgerigar fed sporocysts from opossum 8013. Merozoites were seen in culture on the day of inoculation, and schizonts and merozoites were numerous by day 7 postinoculation (PI). Infected cells were still intact in the original flask infected with *S. falcatula* for at least 4 mo when the culture was cryopreserved. Schizonts and merozoites from this bird were studied ultrastructurally as described by Speer and Dubey (1999).

Sporocysts from 14 of 24 opossums were infective to mice (Table II). *Sarcocystis neurona* schizonts (Fig. 4) were identified mainly tissues of immunodeficient mice fed sporocysts from 8 opossums (Table II).

Sarcocystis speeri (Figs. 5, 6) was identified in tissues of mice fed sporocysts from 8 opossums. In 3 opossums (8047, 8071, 8105) both *S. speeri* and *S. neurona* were present. *Sarcocystis speeri* schizonts were distinguished from *S. neurona* schizonts by the IHC method (Figs. 7, 8). More *S. neurona* and *S. speeri* schizonts were seen in sections stained with specific antibodies than in H&E-stained sections. In H&E-stained sections, schizonts of *S. neurona* and *S. speeri* were difficult to identify, but *S. speeri* schizonts were often larger in size than those of *S. neurona* (Figs. 4, 5).

Sarcocystis neurona was cultured in vitro from the brains of mice fed sporocysts from all 8 infected opossums. These isolates were designated SN-8OP to SN-15OP, based on preliminary observations; all 8 isolates were cryopreserved for further characterization. *Sarcocystis speeri* was not cultured in this study.

Sporocysts from opossum 8026 (reported by Dubey and

TABLE II. Sarcocystosis in immunodeficient mice fed sporocysts from opossums.

Opossum no.	Mouse no. and type*	Day killed or died	*Sarcocystic neurona*			*Sarcocystis speeri*	
			IHC*	Cell culture	Strain designation	Schizonts	Sarcocyst
8026	Many KO and N†	†	—	—		+	+
8030	KO2438, and others‡	DK25	B, Lu +‡§	+ED	SN-8OP	−	−
8047	KO2432, and others‡	DK36	B, Lu	+M617	SN-9OP	+†	+†
8064	N2453	K40	B, Lu			−	−
	KO4010	DK29	B, Lu	+M617	SN-10OP	−	−
	KO4009	K83	—			−	−
8071	N2454	K40	B, Lu			−	−
	KO4011	DK36	B	+M617	SN-11OP	−	−
	KO4012	K83	—	—		−	+
8093	N2462	K40	—			+	+
	KO4013	D36	—			+	+
	KO4014	DK37	—			+	+
8095	N2463	DK34	B	+M617	SN-12OP	−	−
	KO6913	D36	B			−	−
8105	N2464	K40	B			−	−
	KO4015	DK34	B	+M617	SN-13OP	−	+
	KO4016	DK47	—			+	+
	KO2944	K36	—			−	−
8129	KO4003	DK30	B, Lu	+M617	SN-14OP	−	−
	KO4004	DK31	B			−	−
9033	KO5416	D39	—			+	+
	KO5888	DK39	—	—		+	+
	KO5889	K25	—	—		−	−
	KO A	D37	—	—		+	+
	KO B	D37	—	—		+	+
	KO5419	DK40	—			+	−
9046	KO5417	K89	—			−	+
	KO5418	K89	—			−	+
	KO3847	K50	—			−	+
	KO3850	K68	—			−	+
9047	KO5413	K89	—			−	+
	KO5414	K89	—			−	+
	KO3843	K43	—			−	−
	KO3855	K36	—			−	−
9062	KO3844	D16	—			+	−
	KO3851	K16	—			+	−
	KO6100	DK34	—			+	+
	KO6105	DK38	—			+	+
9108	KO5984	D20	B	—		−	−
	KO984	DK24	B	+M617	SN-15OP	−	−
	KO6509	DK32	B			−	−

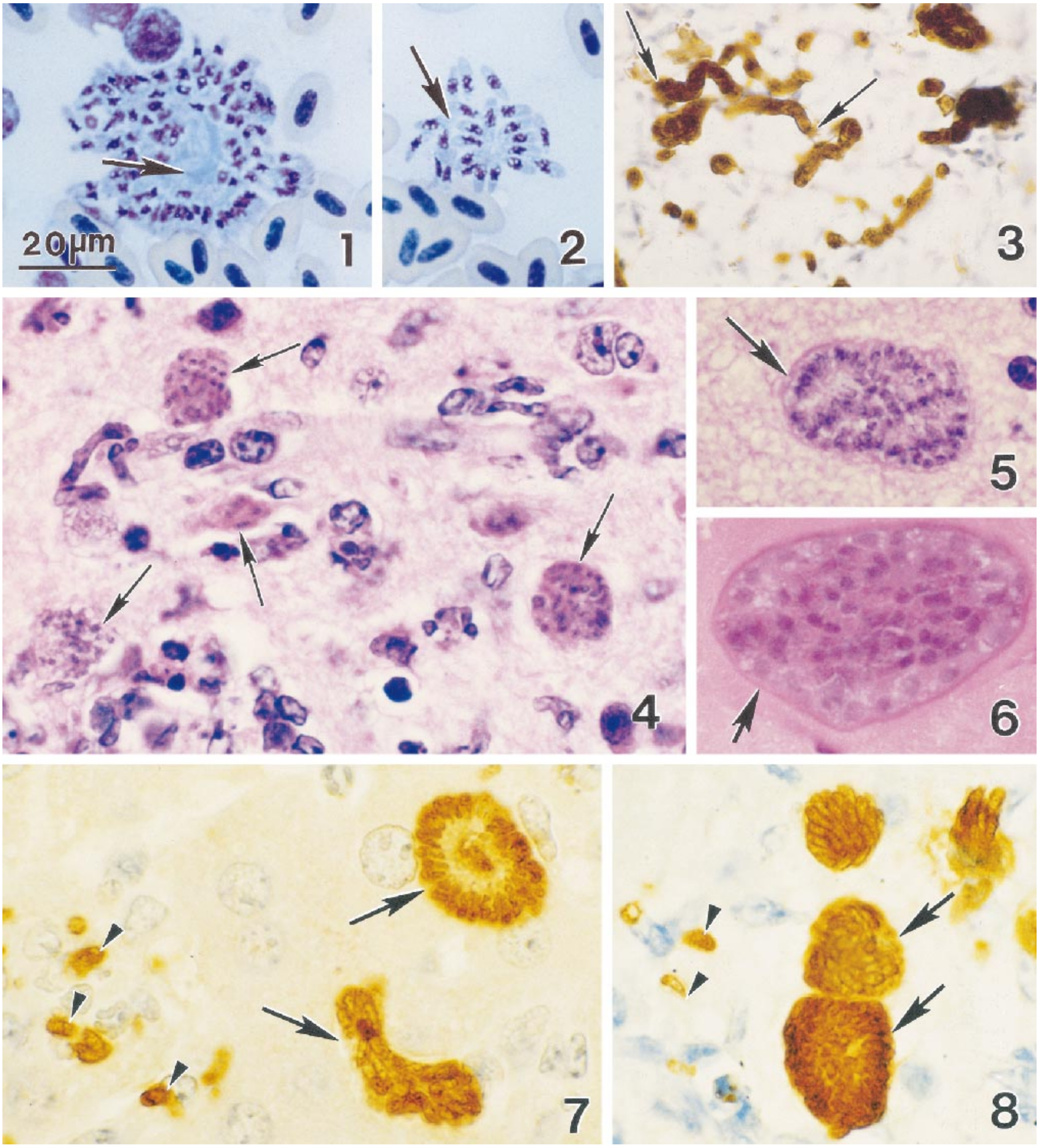
* B = brain, ED = equine dermal cells, KO = knockout, L = lung, M617 = bovine monocytes, N = nude, IHC = immunohistochemistry, D = died, K = killed, DK = killed when ill.
† See Dubey and Lindsay (1998).
‡ See Dubey and Lindsay (1999).
§ In a nude mouse killed day 30, *S. neurona* was found in many tissues, including bronchial epithelia, intestine, spleen, adrenal, liver, kidneys and brain.

Lindsay, 1999) and 8095 (present study) were not infective to birds.

DISCUSSION

Results of this study indicate that *Sarcocystis* sporocysts are widely prevalent (51%) in the North American opossum, *D. virginiana.* It is likely that the prevalence of *Sarcocystis* was underestimated because of the low sensitivity of the methods used. In the present study, intestines were homogenized in 100 ml water, and if no sporocysts were seen in a 10–50-μl aliquot, the sample was considered negative. Thus, light infections would have been missed. Only specimens with microscopically visible sporocysts were bioassayed.

The number of *Sarcocystis* species in opossums is unknown. Until 1995, only *S. falcatula* was thought to be present in opossums. Recent studies indicate that at least 3 species (*S. falcatula, S. neurona, S. speeri*) can be definitely identified by bioassays. The results of the present study indicate that *S. falcatula* is the most prevalent species in the opossum and that organisms infective to budgerigars were considered *S. falcatula*. Whether *S. falcatula* is a single species or mixture of more than 1 species is not known. This question can only be



FIGURES 1–8. *Sarcocystis* stages in budgerigars and mice fed sporocysts from opossums. *Sarcocystis falcatula* in lungs of budgerigars (Figs. 1–3), *S. neurona* schizonts and merozoites in brains of immunodeficient mice (Figs. 4, 8), and *S. speeri* stages in mice (Figs. 5–7). Bar = 20 μm and applies to all figures. **1.** Schizont with budding merozoites. Smear stained with Giemsa. There is no residual body, except for slightly darkly stained cytoplasm (arrow). **2.** Ruptured schizonts with merozoites (arrow). Smear stained with Giemsa. **3.** Section of lung stained with anti-*S. falcatula* antibody. Note tortuous, elongated schizont (arrows). **4.** Brain of nude mouse, 39 DPF. H&E stain. Note several schizonts (arrows) in an inflammatory focus. **5.** Brain of nude mouse 48 DPF. *Sarcocystis speeri* schizont (arrow). H&E stain. This schizont is larger than *S. neurona* schizonts. **6.** Skeletal muscle KO mouse, 64 DPF. Intramuscular sarcocyst of *S. speeri* with thin cyst wall (arrow). H&E stain. **7.** Liver of KO mouse 39 DPF. *Sarcocystis speeri* schizonts (arrows) and merozoites (arrowheads). Immunohistochemical stain with anti-*S. speeri* antibody. **8.** Brain of nude mouse, 39 DPF. Note schizonts (arrows) and individual merozoites (arrowheads). Immunohistochemical stain with anti-*S. neurona* antibody.

resolved when the parasite has been cloned in cell culture and the life cycle completed using cloned isolates. However, our initial attempts to maintain *S. falcatula* continuously in culture for prolonged periods have not been successful. Recent molecular data indicate the heterogenity of *S. falcatula* isolates (Marsh et al., 1999).

Sarcocystis falcatula was named by Stiles in 1893 based on the structure of sarcocysts from the rose-breasted grosbeak (*Pheuticus ludovicianus*). Box and Smith (1982) and Box et al. (1984) studied its life cycle and host spectrum and redescribed *S. falcatula. Sarcocystis falcatula,* unlike other species of *Sarcocystis* (Dubey et al., 1989), was shown to be infective to a variety of birds, namely to cowbirds (*Molothrus ater*), budgerigars (*M. undulatus*), zebra finches (*Poephila guttata*), canaries (*Serinus canarius*), sparrows (*Passer domesticus*), and pigeons (*Columba livia*) but not to chickens (*Gallus gallus*) or guinea fowl (*Numida meleagris*)(Box and Smith, 1982). Recently, *S. falcatula* was cultivated in vitro (Marsh et al., 1997; Lindsay et al., 1999). Lindsay et al. (1999) described schizogonic development of the Cornell strain of *S. falcatula.* The Cornell strain of *S. falcatula* was obtained by feeding muscles of naturally infected cowbirds to an opossum; *S. speeri* and *S. neurona* were not found in the feces of this opossum (J. Dubey, unpubl. obs.). Antibodies produced in rabbits against the culture-derived merozoites of the Cornell strain were used for IHC in the present study. The lack of reactivity of *S. falcatula* sarcocysts to anti-*S. falcatula* antibody is likely due to the stage specificity of the antiserum; the rabbits were immunized with *S. falcatula* merozoites.

The high prevalence of *S. falcatula* in opossums is biologically important for 2 reasons. First, 25 of 44 opossums in the present study were from the 2 zoos. *Sarcocystis falcatula*-like organisms can cause acute sarcocystosis in passerine birds, and several outbreaks have been documented in zoos (Hillyer et al., 1991). Therefore, mortality in birds may not be preventable unless opossums can be eradicated from zoos or at least kept isolated from the aviaries. Second, the high prevalence of *S. falcatula* in opossums interferes with *S. neurona* investigations. One of the definitive measures of the presence of *S. neurona* sporocysts in opossum feces is based on the recovery of *S. neurona* from tissues of immunodeficient mice fed sporocysts (Dubey and Lindsay, 1998). Attempts to fulfill Koch's postulates for *S. neurona* infection in horses so far have been unsuccessful. Fenger et al. (1997) fed sporocysts from opossums to *S. neurona* antibody-negative horses. The horses fed sporocysts developed antibodies specific to *S. neurona,* clinical signs, and lesions consistent with *S. neurona,* but the organisms were not demonstrable in tissues of the horses (Fenger et al., 1997). Recently, *S. falcatula* was found to be not infective to horses (Cutler et al., 1999).

Using recently developed genetic markers, Tanhauser et al. (1999) reported the existence of 3 or more species of *Sarcocystis* in opossums. Of the 9 inocula from the intestines of wild-caught opossums from Florida, 5 were considered to be *S. neurona,* 2 as *S. falcatula,* and 2 were different from *S. falcatula* and *S. neurona* (Tanhauser et al., 1999). In the present study, *S. falcatula* was found in 21 of 23 inocula with visible sporocysts. Without knowing the sensitivity and specificity of the methods used, it is not reasonable to compare results of these 2 studies.

The results obtained in the present survey are qualitative with respect to *S. neurona* and *S. speeri* infections. First, not all samples were tested in KO mice. *Sarcocystis speeri* rarely forms sarcocysts in nude mice (Dubey and Lindsay, 1999). Thus, definitive identification of *S. speeri* might have been missed in samples fed only to nude mice or when KO mice died before 7 wk PI. Sarcocysts of *S. speeri* were evident in muscles of KO mice >30 DPF (Dubey and Lindsay, 1999). Second, the threshold of sporocysts that can cause histologically demonstrable sarcocystosis by the oral route in nude or KO mice is unknown. Even the number of *S. neurona* merozoites that can cause sarcocystosis after parenteral inoculation is unknown. Nude mice injected with 10,000 or more cultured merozoites developed histologically demonstrable *S. neurona* infections (Marsh et al., 1997; Dubey and Lindsay, 1998). *Sarcocystis speeri* merozoites are not infective to KO mice by the subcutaneous route (Dubey et al., 2000).

The diagnosis of equine protozoal myeloencephalitis (EPM) in horses is based on clinical signs and the detection of *S. neurona* antibodies in western blots using culture-derived *S. neurona* merozoites. Such data are not available to distinguish serologically *S. neurona* and *S. speeri* infections in mice. The immunohistochemical method used to distinguish *S. neurona* from *S. speeri* is insensitive and not likely to detect the presence of <10,000 organisms per/1 g of tissue (J. Dubey, unpubl. obs.). Therefore, there is a need to determine the minimal infective dose of *Sarcocystis* for birds and mice.

In the present study, sporocysts were not concentrated in the inoculum by digestion in 5.25% sodium hypochlorite (bleach). Whereas bleach kills bacteria and dissolves host tissue, many sporocysts are lost by the washing procedures used to remove chlorine. I am not aware of any study comparing the infectivity of bleach-treated versus untreated *Sarcocystis* sporocysts. Although no quantification was performed, *S. speeri* sporocysts remained infective for mice after 16 mo storage in antibiotics at 4 C.

The identification of *S. neurona* and *S. speeri* in the present study was based on conventional histology and IHC. Both species of *Sarcocystis* are infective to nude and KO mice. Schizonts of both species may be found in several organs but are mostly in the brain. In H&E-stained sections, *S. neurona* schizonts and merozoites are smaller than those of *S. speeri* (Figs. 4, 5), and thus *S. speeri* schizonts may be visible at 400×, whereas *S. neurona* schizonts are rarely visible at the same magnification. IHC can clearly distinguish schizonts of *S. neurona* from those of *S. speeri* (Figs. 7, 8). Tissue localization may also vary with the type of the mouse used. For example, *S. speeri* produces more schizonts in the liver of nude mice than in the liver of KO mice and *S. neurona* does not form sarcocysts in nude or KO mice. *Sarcocystis speeri* forms sarcocysts in KO mice but rarely in nude mice (Dubey and Lindsay, 1999).

Sarcocystis neurona is more easily grown in cell cultures than *S. speeri* (J. Dubey, unpubl. obs.). In the present study, all attempts to culture *S. speeri* from the brains of infected mice were unsuccessful, whereas using the same procedures, *S. neurona* was cultured from 8 opossums. However, *S. speeri* merozoites and schizonts have been cultured from the 2 Argentinian opossums (Dubey et al., 2000).

The isolation of *S. neurona*-like organisms from the brains of mice fed sporocysts from opossums were tentatively designated as *S. neurona* isolates SN-8 to SN-15P (P for opossums) following the terminology used earlier (Dubey, Mattson et al., 1999). Previous reports of *S. neurona* isolates from EPM cases from horses were recently summarized (Dubey, Mattson et al., 1999).

Sarcocystis neurona is widely prevalent in horses in the Americas. Antibodies to *S. neurona* were found in 53.6% of 1,056 horses from Ohio (Saville et al., 1997), 45.3% of 117 horses from Pennsylvania (Bentz et al., 1997), 45% of 334 horses from Oregon (Blythe et al., 1997), and 35.5% of 76 horses from Argentina, and 36% of 101 horses from Brazil (Dubey, Kerber et al., 1999; Dubey, Venturini, Venturini, McKinney, and Pecoraro, 1999). The only known mode of transmission of *S. neurona* to horses is via the ingestion of food and water contaminated with sporocysts from opossum feces, and epidemiologic data support this conclusion. *Sarcocystis neurona* infection in horses has not been reported outside the range of opossums in the Americas. Although *S. falcatula* and *S. speeri* have been reported in the South American opossum (*Didelphis albiventris*) (Dubey and Lindsay, 1999; Dubey, Venturini, Venturini, and Speer, 2000), *S. neurona* has not yet been found in opossums from South America.

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